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Hjorth, Rune; Baun, Anders

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DTU Environment
Department of Environmental Engineering

Technical University of Denmark

Ecotoxicity testing of iron nanoparticles utilized for remediation

Rune Hjorth and Anders Baun
Technical University of Denmark, Department of Environmental Engineering, DK-2800 Kgs. Lyngby, Denmark
Corresponding author: ruhj@env.dtu.dk

Intro

Nanoparticles engineered to remediate polluted soil and groundwater constitute a potential risk to the environment as they have an intended environmental release in high quantities. In this study, a range of iron nanoparticles developed for remediation purposes were tested in ecotoxicity tests with algae (Pseudokirch*neriella subcapitata*), crustacean (*Daphnia magna*) and bacteria (*Vibrio fischeri*) in concentrations between 10 – 1,000 mg/L.

Methods

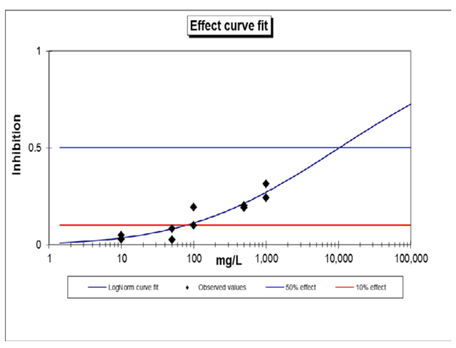
Three tests were used for ecotoxicity testing:

- Algal 48h growth Inhibition Test (OECD 201)
  - Biomass determined spectrophotometrically
- Daphnia 48h immobilization Test (OECD 202)
  - Visual inspection of mobility
- Luminescent 15 min Bacteria Test (ISO 11348-3)
  - Light emission (luminescence) measured photometrically

Result

Bacteria

Normal setup



- Most particles initially showed partial inhibition above 100 mg/L.
- Possible interference due to nanoparticle shading .

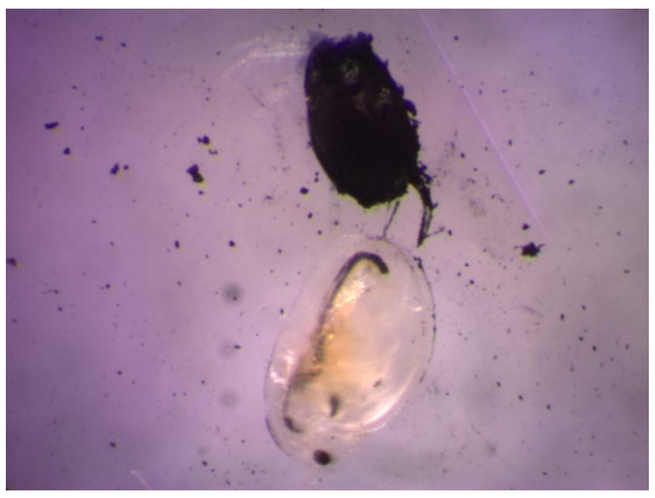
Double-tube setup – the effect of shading

- Shading from nanoparticles confirmed to reduce light emission in a double-tube setup.
- In this setup, the bacteria were kept in one tube and the nanoparticles in a separate tube surrounding the bacteria.

Results

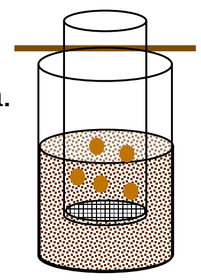
Nanoparticle shading at high concentrations interfered with the test setup, but further experiments showed that the effect can be accounted for through the use of an internal standard for light emission.

Daphnia



Results

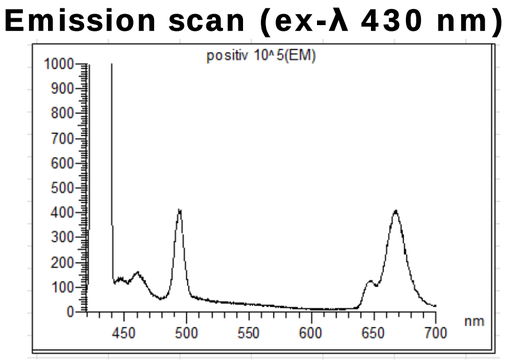
The nanoparticles tended to sorb to the exoskeleton of the daphnia.



- Inhibition of movement was observed, seemingly due to physical effects, not toxicity.
- As suggested by Sørensen et al. (2014) future investigations can be done in a separation test without contact between daphnia and aggregated and sedimented nanoparticles to distinguish between physical or chemical/toxic effects.

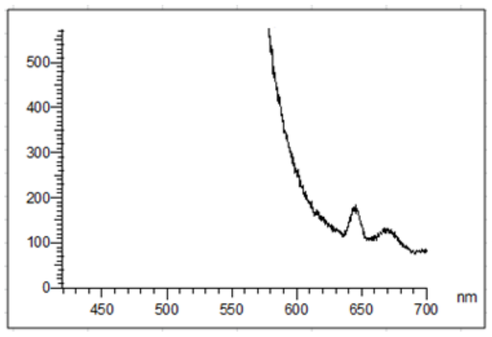
Algae

Emission scan (ex-λ 430 nm) of algae



- Chlorophyll is measured spectrophotometrically using excitation wavelength at 430 nm and quantifying the emission of fluorescence at wavelength 671 nm.

Emission scan (ex-λ 430 nm) of algae + nanoiron (500 mg/L)



- As seen in the emission scan, the peak at wavelength 671 nm is influenced by the presence of the nanoparticles.
- To counter this interference, calculation of the peak area at 671 nm can alternatively determine the biomass.

Results

- Evaluation of results from algae testing have to be done carefully due to spectrophotometric nanoparticle interference.

Conclusion & Improvements of test design

- Initial results showed signs of toxicity at higher concentrations, however, further investigations proved that the turbidity of the tested suspensions was responsible for most of the apparent toxicity.
- All suspensions were cloudy and unstable in test media and the high degree of agglomeration and sedimentation affected the outcome of the tests by interfering with the measuring principles of the tests, e.g. the mobility of the daphnia, chlorophyll fluorescence and bacterial luminescence.
- Adjusting the methods to account for this interference, for instance by utilizing internal standards for light emission in the bacterial test was shown to diminish the risk of generating false positive results.

Take-home message

While testing concentrations up to 100 mg/L is relevant for hazard identification and classification purposes, testing iron nanoparticles at higher concentrations not only decreases the environmental relevance but also increases the influence of physical effects such as turbidity and concentration-dependent agglomeration. Therefore, the main focus should be on concentrations <100 mg/L and care should be taken when conducting ecotoxicological testing of iron nanoparticles at higher concentrations.

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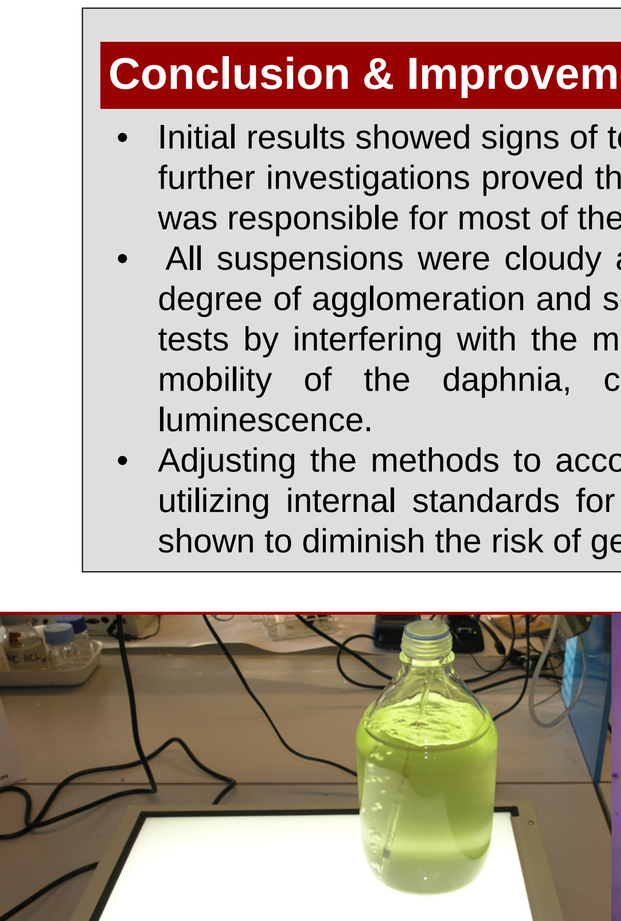

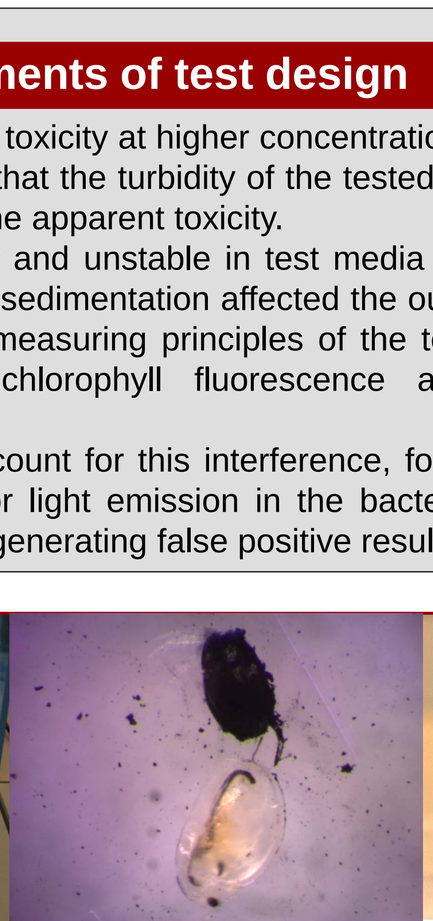

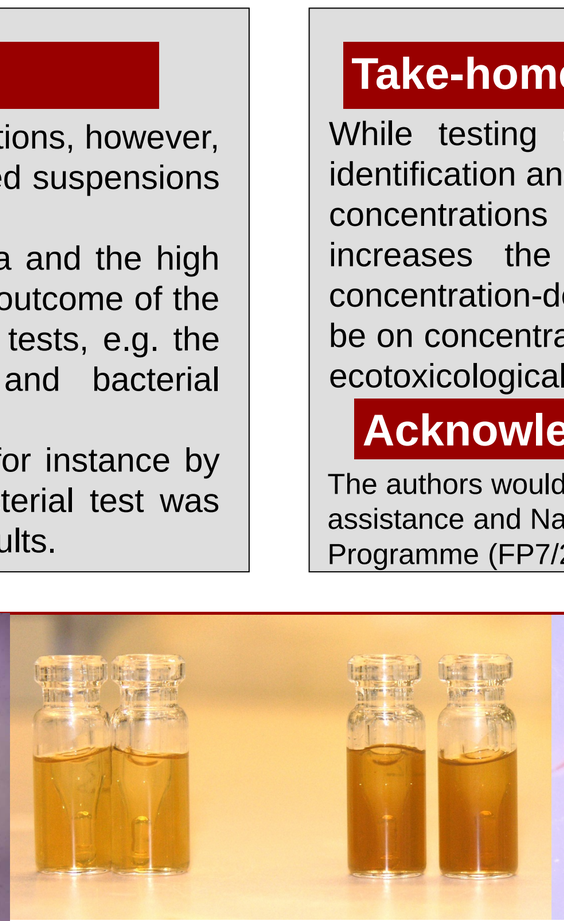

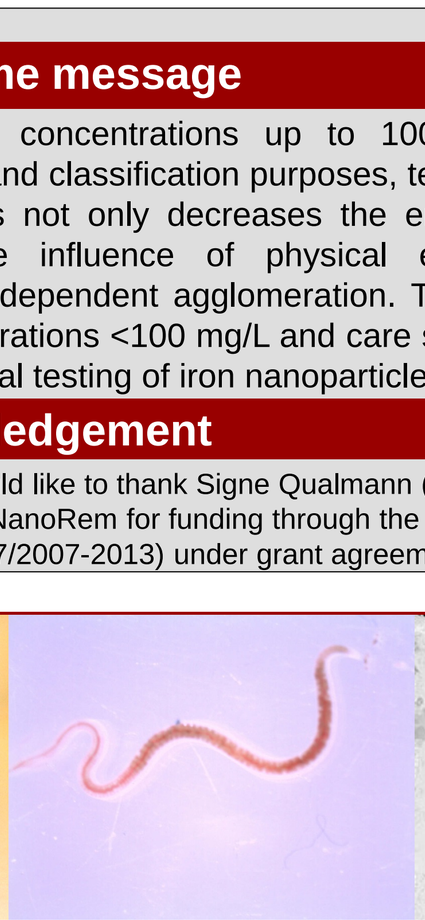

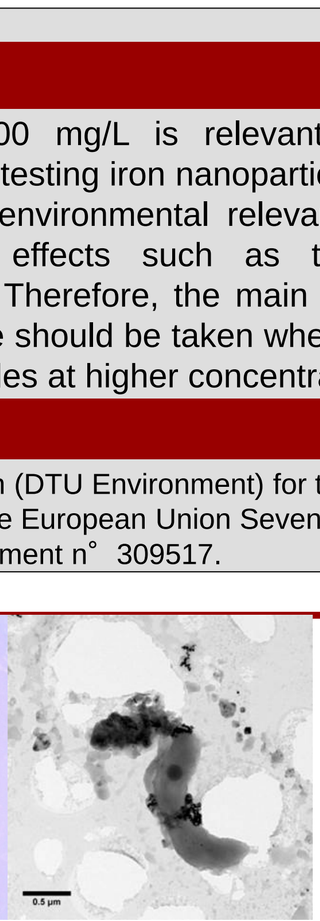

Sara N. Sørensen, Cristina Giron Delgado, Christian Engelbrekt and Anders Baun (2014), Platinum NP toxicity in freshwater algae and crustaceans - A physical or chemical effect?, Platform Presentation 24th Annual SETAC Europe, Basel, Switzerland.